Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

A METHOD FOR THE DETECTION OF PHENOLS PRODUCED BY BACTERIA

From the Department of Bacteriology and Hygiene, University of Cincinnati.

WILLIAM H. BELL

The determination of phenols produced by the metabolic activities of organisms deserves consideration, since an accurate and sensitive method would furnish an additional means of distinguishing groups of micro-organisms whose cultural and morphologic characteristics are similar.

The method ordinarily used for the detection of phenol is essentially as stated by Eyre.[1] The culture is prepared in a flask containing nutrient broth and incubated. After incubation, 5 c c of 25% sulphuric acid is added to the cultivation, and the flask is connected with a condenser; 15 to 20 c c are distilled over, and the distillate divided into 3 portions, (a), (b) and (c). To (a), 0.5 c c of Millon's reagent is added and the solution is boiled; red color = phenol. To (b), 0.5 c c ferric chlorid solution is added; violet color = phenol. (If the distillate be acid the reaction will be negative.) To (c), bromine water is added; crystalline white precipitate of tribromphenol = phenol. In case indol and phenol appear to be present in cultivations of the same organism, it is recommended that they be separated before testing. This is done by redistilling the first distillate (which would contain both indol and phenol) after rendering it alkaline with caustic potash. The distillate contains indol, the residue phenol. The residue is saturated with CO_2 and redistilled. The last distillate will contain phenol and is tested as stated in the foregoing.

Rettger,[2] in his study of the chemical products of B. coli and B. lactis-aerogenes, used a somewhat similar method. The medium used in his experiments was an egg-meat mixture, and incubation was carried on in the presence of hydrogen. Portions of 400 c c of the decomposition mixture were diluted with an equal volume of water, and after

Received for publication June 7, 1921.

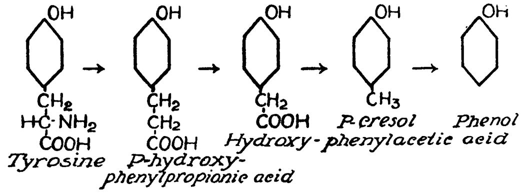
---

[1] Bacteriological Technique, 1916, p. 287.

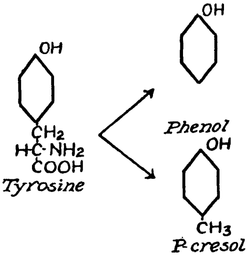
[2] Am. Jour. of Physiol., 1903, 8, p. 284.

the addition of 5 c c of dilute sulphuric acid, were distilled with steam until from 600 to 700 c c of distillate had formed. The distillate was made alkaline with KOH, and again distilled with steam until 500 c c of liquid had collected. Indol and skatol were in the distillate. The last distillation residue was saturated with CO_2 and distilled until 500 c c of liquid had collected. Phenols were in this distillate.

The mother substance of phenol is tyrosine, possibly phenylalanine or other aromatic amino acids, if any exist, other than tryptophane. The exact manner in which tyrosine is decomposed in putrefaction is not certainly known. The transformation, as suggested by Matthews,[3] may be as follows:



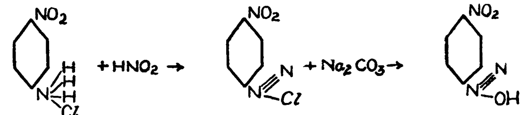
It is quite possible that tyrosine is split directly to yield phenol or p-cresol:



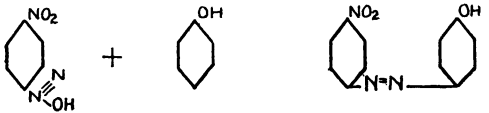
The method proposed in this paper is dependent on the formation of an azo-dyestuff, brought about by the reaction of a diazotized aromatic amine and a phenol in alkaline solution. The formation of such a colored compound affords an extremely sensitive method for the detection of minute quantities of phenol. In considering a suitable amine for coupling with the phenol, paranitraniline was chosen, since diazotized paranitraniline will couple practically quantitatively and instantaneously, especially so in dilute solutions. The reaction is as follows:

---

[3] Physiological Chemistry, 1916, p. 745.

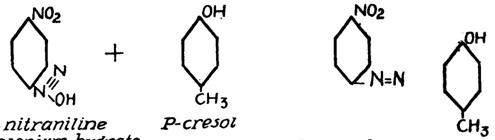


P-nitraniline HCl — P-nitraniline diazonium chloride — P-nitraniline diazonium hydrate



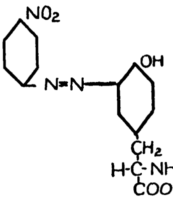
P-nitraniline diazonium hydrate — Phenol — *P-nitrobenzene azo phenol*

With P-cresol, we would have:



P-nitraniline diazonium hydrate — P-cresol — *P-nitrobenzene azo p-cresol*

An important advantage of this test is that indol will not couple with diazotized paranitraniline. It is important to note that tyrosine is capable of uniting with a diazotized aromatic amine to form azo dyestuffs. With paranitraniline, the resulting dyestuff would have the following constitution:



P-nitrobenzene azo tyrosine

Phenylalanine, on the contrary, would not be capable of such union, since it is neither an aromatic amine nor a phenol.

From these facts, it is evident that phenol or p-cresol could be detected in a bacterial culture by resorting to distillation of the acidified culture with steam, and after alkalinizing the distillate, adding diazotized paranitraniline and noting the formation of the azo dye. As stated the presence of indol in the distillate would not interfere.

The procedure recommended in applying the test is as follows:

1. Cultivation: The organism to be tested is grown, most conveniently in a 1 liter flask, on a suitable medium, such as nutrient bouillon, egg-meat mixture, egg-bouillon, etc., incubation being carried on under aerobic, anaerobic or partial tension conditions. After incubation, the culture is ready for distillation.

2. Distillation: The flask containing the culture is connected up with a steam distillation apparatus. In order to avoid any mechanical carrying-over of medium, the apparatus was set up as shown in fig. 1.

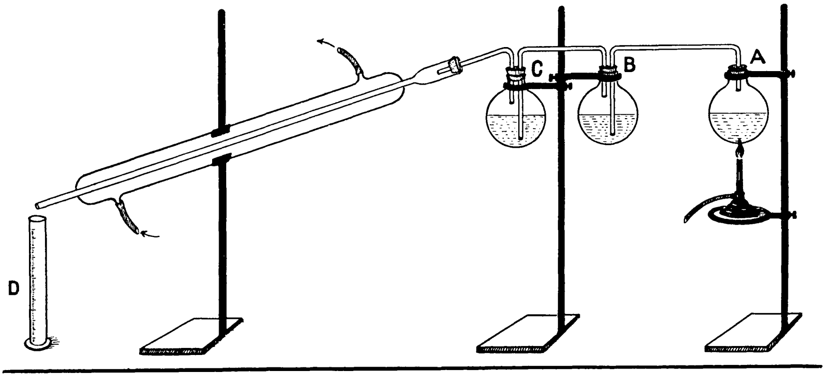


Fig. 1.—The flask was used to furnish steam for the distillation. The steam evolved caused the medium in B, which had been acidified previously with sulfuric acid, to boil, thus producing steam which in turn carried phenols over into C. C contained distilled water, acidified with sulfuric acid. The steam evolved from B caused the water in C to boil, the distillate containing phenols being collected at D. The distillate was made distinctly alkaline by the addition of 10% sodium carbonate.

3. Preparation of Diazo Solution: One-tenth gm. of pure paranitraniline is placed in a test tube and 0.6 c c concentrated HCl added, heat being applied to hasten solution. The solution is then diluted to 5.0 c c with distilled water and 1.0 c c of M/1 $NaNO_2$ added. The solution is allowed to stand for 5 minutes; then it is added drop by drop to the distillate, care being taken that the distillate is kept alkaline. The appearance of a red color indicates phenols. In extreme dilutions the color appears yellowish-red. The observer must not be deceived by the formation of a light yellow color, as this is due to paranitraniline diazonium hydrate.

This method will show on blank determinations, a distinct yellowish-red color with phenol diluted 1 part to 1,000,000. In culture mediums, it is easily possible to show the presence of phenol in concentrations of 1 : 500,000. The sensitiveness of this reaction is especially valuable, when we consider that the reagents heretofore used will not show their characteristic colorations in dilutions as low as 1 : 10,000. Furthermore, the presence of other compounds, such as indol, interferes with ferric chloride and Millon's reagent, necessitating additional work in separating the two before testing. As tyrosine and derivatives of tyrosine, in addition to phenol and p-cresol, will couple with the formation of an azo dye similar to those formed by phenol and p-cresol, it is important to state that tyrosine and, so far as known, split-products of tyrosine, other than phenol and p-cresol, will not distill over with steam.

In the near future, a quantitative method based on the above will be given, also experiments involving phenol production by various species of bacteria.